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REVIEW

Cellular mechanisms and local progenitor activation to regulate skeletal muscle mass

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Abstract Skeletal muscle hypertrophy is a result of increased load, such as functional and stretch-overload. Activation of satellite cells and proliferation, differentiation and fusion are required for hypertrophy of overloaded skeletal muscles. On the contrary, a dramatic loss of skeletal muscle mass determines atrophy settings. The epigenetic changes involved in gene regulation at DNA and chromatin level are critical for the opposing phenomena, muscle growth and atrophy. Physiological properties of skeletal muscle tissue play a fundamental role in health and disease since it is the most abundant tissue in mammals. In fact, protein synthesis and degradation are finely modulated to maintain an appropriate muscle mass. When the molecular signaling is altered muscle wasting and weakness occurred, and this happened in most common inherited and acquired disorders such as muscular dystrophies, cachexia, and age-related wasting. To date, there is no accepted treatment to improve muscle size and strength, and these conditions pose a considerable anxiety to patients as well as to public health. Several molecules, including Magic-F1, myostatin inhibitor, IGF, glucocorticoids and microRNAs are currently investigated to interfere positively in the blueprint of skeletal muscle growth and regeneration.

Keywords Muscle hypertrophy · AKT · Magic-F1

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Introduction

The basic contractile unit of skeletal muscle is the muscle fiber; thousands of muscle fibers form together an individual skeletal muscle. Muscle fibers are limited by a plasma membrane (sarcolemma) surrounded by a basal lamina, and outside of this, a connective tissue composed by scarce ECM (extra cellular matrix) proteins, capillaries and nerve terminals (Buckingham 2001). Muscular hypertrophy and atrophy are two opposite and mechanistically linked phenomena regulating muscle cell size, finally determined throughout a balance between new protein accumulation and degradation of pre-existing proteins (Sandri 2008). In response to exogenous stimuli or to biological factors such as age or nutrition, the muscle is able to adapt by increasing the size and amount of contractile proteins (Fig. 1). This leads to increase in fiber size and their consequent force production. Muscle remodeling occurs throughout the entire life although at different rate considering the developmental stages. During embryo formation and childhood, upregulation of protein synthesis is accompanied by a cellular turnover, in which satellite cells incorporate into new muscle growing fibers. In adult muscle, cellular turnover is strongly reduced and the physiological conditions leading to muscle growth are basically determined by increasing synthesis and downregulating protein turnover (Schiaffino et al. 2007). Basically, two different forms of hypertrophy exist: sarcoplasmic and myofibrillar; the first is characterized by an increase in the sarcoplasmic volume with no accompanying increase in muscular strength. During myofibrillar hypertrophy, the myofibrils increase in number and add muscular strength as well as a small increase in muscle size. Sarcoplasmic hypertrophy is characteristic of bodybuilder muscles while myofibrillar hypertrophy is characteristic of weightlifters. In stark contrast, muscle atrophy

also called muscle wasting is the result of contractile protein loss with a reduction in fibers cross sectional area and consequently in muscle growth. At the molecular level, signals controlling muscle growth or atrophy are different but finely interconnected (Fig. 1), and the biochemical pathways convert on a common set of molecules regulating protein synthesis or breakdown (Sartorelli and Fulco 2004). Nonetheless, these two processes basically hold the key to understanding the mechanism involved in the regulation of skeletal muscle growth. Recently, stem cell therapy approaches are rapidly developing to counteract muscle degeneration mainly by exogenous cell delivery or endogenous activation of muscle progenitors (Sampaolesi et al. 2005). Those techniques also result in muscle mass increase providing new, functional fibers to the damaged muscle (Rudnicki et al. 2008). In this review, molecular mechanisms regulating muscle growth will be elucidated, briefly describing the major determinants and further considering the role of satellite cells in promoting muscular hypertrophy. Finally we also take into account the effect of exogenous stem cell delivery and endogenous stem cell activation on skeletal muscle mass and architecture.

Magic-F1 and muscle remodeling

Magic-F1, which name stays for cMet Activating Genetically Improved Chimeric Factor-1, is a recombinant protein containing a tandem repetition of the Hepatocyte Growth Factor (HGF) domains mainly involved in the cMet activation (Cassano et al. 2008). While during development HGF/cMet signaling mediates homing of progenitors cells, in adulthood is responsible of activation and early proliferation phase of satellite cells. Although satellite cells are a functionally heterogeneous population, it is widely believed that those cells are the committed stem cells of adult skeletal muscle tissues. Then, the signal is shut off to allow myogenic differentiation, since HGF promotes proliferation by inhibiting the differentiation program. By contrast, Magic-F1 displays a selective pattern of biological activities qualitatively different from its native factor. Magic-F1 supports Protein kinase B (PKB/Akt) but not Mitogen Activated Protein Kinase (MAPK) phosphorylation, thereby inhibiting proliferation of skeletal muscle progenitors though retaining the ability to stimulate the anti-apoptotic cascade. Magic-F1 protects myogenic precursors against apoptosis, thus increasing their fusion ability and enhancing muscular differentiation. Myogenic cell line C2C12 expressing the recombinant factor showed a faster differentiation compared with mock, together with an early upregulation of master genes such as MyoD and Myf5. In vivo, muscle hypertrophy and cell survival are elicited following Magic-F1 expression; transgenic mice expressing the recombinant protein in a muscle specific

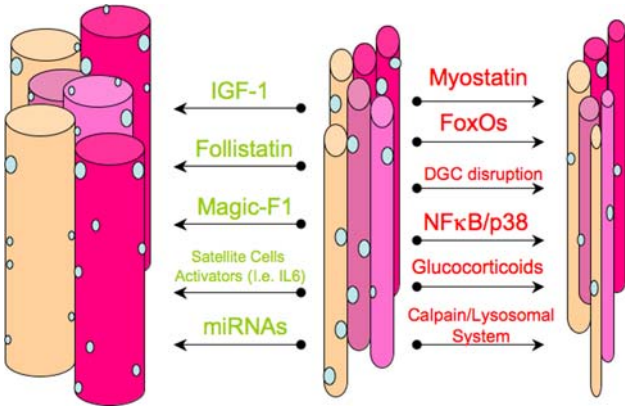

Fig. 1 Muscle remodeling is a complex interplay among many key factors, controlling both protein synthesis and satellite cells activation. As a final result, fiber size and nuclear content can be increased (hypertrophy, *left panel*) or strongly reduced (atrophy, *right panel*) Molecules positively (*left arrows*) and negatively (*right arrows*) affecting fiber size are indicated

fashion show muscle hypertrophy along with faster regeneration after injury and increased capillary formation. Together with that, a better running performance and muscular strength was observed in transgenic mice. Crossing of Magic-F1 transgenic mice (Fig. 1) with α-sarcoglycan knock-out mice –a mouse model of muscular dystrophy– or adenovirus-mediated Magic-F1 gene delivery resulted in amelioration of the dystrophic phenotype as measured by both anatomical/histological analysis and functional tests. Because of these features, Magic-F1 represents a novel factor able to dissociate the mitogenic effect of HGF from its anti-apoptic properties and a good candidate in counteracting wasting and degeneration in muscular diseases such as muscular dystrophy. Given its small size, it could be used as an adjuvant transgene to improve homing and efficacy of gene and cell therapy protocol (Fig. 1).

IGF-1 pathways

Insulin Growth Factor-1 (IGF-1) is among the best characterized muscle growth promoting factors, produced mainly in the liver under the control of the Growth Hormone (GH) but its expression is located also in the skeletal muscle, suggesting a paracrine/autocrine role of IGF-1 in positively regulating muscle growth. Different isoforms of IGF-1 exist thanks to different RNA spliced variants. Human skeletal muscle has been found to express at least two isoforms (Hameed et al. 2004). These are IGF-1Ea, which is the liver type or systemic form and IGF-1Ec, also called Mecano Growth Factor (MGF), an autocrine/paracrine form that is particularly interesting as it is expressed in response to mechanical stimuli and cellular damage.

Increased muscle loading results in augmented expression of the IGF-1 encoding gene both in humans and animal models (DeVol et al. 1990; Bamman et al. 2001). MGF expression peaks earlier than IGF-1Ea and after damage or mechanical stress is produced as a pulse lasting only 2 days, then replaced by a longerlasting expression of IGF-1Ea (Haddad and Adams 2002; Hill and Goldspink 2003; Haddad and Adams 2004).

The latter factor is likely to be involved in maintaining protein synthesis to complete the muscle trophism, furthermore IGF1Ea can promote both proliferation and differentiation of satellite cells (Allen and Boxhorn 1989; Doumit et al. 1996; Adi et al. 2002). IGF-1Ea induced muscle hypertrophy is accompanied by an increase in DNA content, centronucleated fibers and upregulation of different myosin isoforms; supporting the involvement of satellite cells in IGF-1 signals (Adams and McCue 1998; Fiorotto et al. 2003). Muscle specific overexpression of IGF-1Ea in transgenic mice results in muscle hypertrophy and increased regeneration during senescence, together with a corresponding increase in physiological muscle strength (Coleman et al. 1995; Musaro et al. 2001). IGF-1 isoforms act through direct interaction with their own receptor IGFR-1, a tyrosin-kinase leading to the final activation of the AKT by the generation of phosphatidylinositol-3,4,5-triphosphates (PIP3). Phosphatidylinositol-3 Kinase (PI3Kinase) and the phosphatases PTEN and SHIP2 represent the first checkpoint of this pathway by inversely regulating the formation of PIP3, which recruits AKT on the plasma membrane allowing the direct phosphorylation by PDK1 and the mTOR-Rictor complex. In mammals, there are three different AKT genes: AKT1 (PKBα), AKT2 (PKBβ) and AKT 3 (PKBγ) involved in different functions; in skeletal muscle AKT1 seems to be the responsible for mediating the muscle growth and protein upregulation signals (Yang et al. 2004). Two major AKT downstream molecules relevant for muscle remodeling are the mechanistic target of rapamycin (mTOR), activated by AKT, and glycogen synthase kinase 3β (GSK3β), inhibited by AKT. Forkhead transcription factors (FoxOs) represent the third mediator of this pathway, crosslinking the synthesis protein machinery with the muscle atrophy program. Basically GSK3β blocks protein translation by inhibiting the eIF2B factor, so probably GSK3β participates in muscle growth processes in a mTOR-independent fashion, also if it is still debated if its inhibition is sufficient to induce per se muscle hypertrophy (Hardt and Sadoshima 2002). mTOR is selectively blocked by the immunosuppressant rapamycin and this complex is composed by two distinct elements: mTORC1, which contains Raptor, is rapamycin sensitive and acts on S6 K and 4EBP1 signaling. It is still unclear if S6 K is activated only by mTORC1 or if other molecules could trigger it but might be that, before the final activation by mTORC1, S6 K need to be primed by direct phosphorylation of PDK1 (Saitoh et al. 2002; Hannan et al. 2003). In addition, mTORC1 targets directly the inhibition of 4EBP-1 (called also PHAS-1), which is a negative regulator of the eIF-4E initiation factor; this system is thought to be the main route through which the mTOR complex induces protein synthesis and muscle growth. Thus, mTORC1-mediated inhibition of 4EBP1 together with the inhibition of GSK3β results in the activation of eIF-4E and eIF-2B, respectively, with subsequent increase in protein synthesis and muscle mass. The mTORC2 complex, which contains Rictor, is more involved in the atrophic pathway by regulating in turn the AKT-FoxOs complex. AKT plays a key role in modulating the atrophic pathway by cytoplasmic retention and inactivation of FoxO proteins, mediated by the mTORC2 complex (Tran et al. 2003; Sandri et al. 2004) (Fig. 2). FoxO proteins are very conserved transcription factors originally discovered as genes overexpressed in rabdomyosarcoma and tumorigenic processes (Greer and Brunet 2005). In vitro, C2C12 cells overxpressing FoxOs showed a strong inhibition of the differentiative events, by contrast silencing of FoxOs results in an enhanced differentiation associated with myosin upregulation (Hribal et al. 2003). In vivo, constitutive activation of FoxOs protein causes a dramatic atrophy of myotubes and mature muscle fibers. In muscle, three different isoforms of FoxO proteins exist, namely called FoxO1, FoxO3a and FoxO4. During muscle atrophy FoxOs expression level are increased and reduced AKT activity induces dephosphorilation of these factors and their consequent activation. Moreover, FoxOs can in turn upregulate 4EBP1 and inhibit Raptor and mTOR, providing another mechanism of skeletal muscle atrophy (Southgate et al. 2007). Because FoxOs play a key role in activating the atrophic program thanks to the atrogenes Muscle Ring

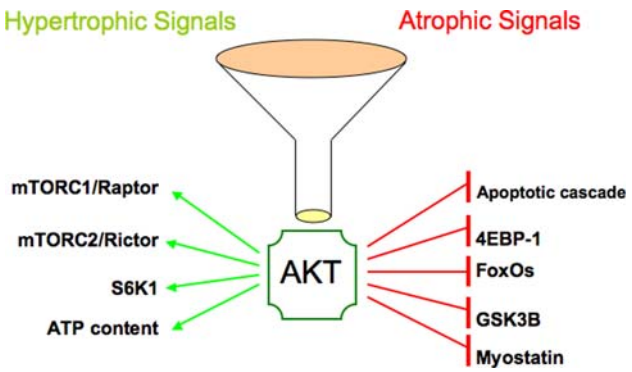

Fig. 2 AKT plays a dual role in muscle remodeling. At molecular level, AKT represents the main crosstalk between hypertrophic and atrophic pathway. Positive (*left side* of the panel) and negative (*right side* of the panel) regulators of its activity are indicated. Following activation, AKT acts by either activating or blocking molecular targets controlling cell metabolism and growth

Finger 1 (MURF1) and muscle atrophy F-box (MAFbx, also known as Atrogin-1), inactivation of these pathways finally prevent atrophic events. Transgenic mice expressing FoxOs in a muscle specific way show reduced body weight and dry mass together with smaller fiber size (Kamei et al. 2004). A second atrophic FoxO-independent system downstream of AKT cascade has been recently discovered by the isolation of a group of genes sensitive to dexamethasone treatment and inversely regulated by IGF-1 treatment, pointing out the existence of multiple checkpoints of atrophy signaling downstream of PI3 K/AKT cascade (Latres et al. 2005). The crosstalk network between protein degradation and muscle growth is complicated and involved not only AKT but also downstream proteins such FoxOs and a fine tuned balance of these pathways is needed to guarantee a correct muscle mass (Fig. 2).

Atrophy and atrogenes

Skeletal muscle atrophy is characterized by a decrease in the size of pre-existing muscle fibers and is observed in many physiological and pathological conditions such as microgravity, critical illness, HIV, cancer cachexia and aging (Sever et al. 1996; Miro et al. 1997; Baracos 2001; Mitch and Price 2001; Singh et al. 2001; Adams et al. 2002, 2003; Di Giovanni et al. 2004; Price et al. 2006). It has been demonstrated that muscle atrophy is regulated by the crosstalking of well known pathways such as the calpain system, the lysosomal and the ubiquitin–proteasome pathways. All these systems are responsible for the proteolysis of structural muscle proteins, leading to muscle wasting (Voisin et al. 1996; Huang and Forsberg 1998; Lecker et al. 1999). A major contribution in understanding the process of atrophy came from the pioneeristic study focused on the molecular behavior of key molecules such as MAFbx and MURF1 (Bodine et al. 2001; Gomes et al. 2001). Using differential display and complementary cDNA microarray approaches coming from different animal models of atrophy such as diabetes, cancer cachexia, denervation, these two genes were identified to be overexpressed compared to control animals. These two genes correspond to ubiquitin-protein ligases expressed solely in skeletal and cardiac muscle that mediate the processes of muscle remodeling and increase proteolysis of disuse atrophy. For instance, MURF1 was firstly identified as the ubiquitin-ligase responsible for the direct binding and degradation of titin, a huge myofibrillar protein, and myosin heavy chain (McElhinny et al. 2002; Clarke et al. 2007). Further confirm of the functional role of these atrogenes came from the experiments performed on MAFbx-/- and MURF1-/- transgenic mice, refractory to muscle-loss phenomena induced by denervation. Importantly, during normal muscle homeostasis there are no evidences of muscle alteration in the transgenic mice, suggesting that those genes do not play a key role in physiological processes. These finding uncover that atrophy is not simply a "loss of hypertrophy" but a real active process controlled by specific signals and biochemical pathways. Nonetheless, muscle atrophy program is linked with hypertrophic pathways. As mentioned in the previous section, IGF-1Ea activation blocks through the AKT-FoxO complex the accumulation of MAFbx transcripts and prevents proteolysis events in dexamethasone-induced atrophy animal model (Sacheck et al. 2004). Animal models of muscular atrophy treated with intramuscular injection of IGF-1 show no increase in MAFbx and MURF1 mRNA and the benefit observed in treated animals is mostly due to the dephosphorylation and reduced activation of FoxO1 and FoxO3a. Moreover, FoxOs can downregulate both Raptor and mTOR and upregulate directly 4EBP1. These results clearly indicate that the IGF-1-AKT pathway prevents atrophy operating through the FoxO proteins (Sandri et al. 2004; Stitt et al. 2004; Southgate et al. 2005). In certain pathological conditions, nuclear Factor κB (NF-κB) represents another factor involved in muscle wasting by mediating the effects of Tumor Necrosis Factor α (TNFα) and Interferon γ (IFN-γ), however, NF-κB seems not linked to MAFbx and MURF1 signaling, since NF-κB overexpression doesn't interfere with MAFbx expression (Li et al. 2003; Sandri et al. 2004; Southgate et al. 2007). In vitro studies on C2C12 system suggest that blockade of NF-κB avoids protein loss content and postulate that probably the TNFα-induced inhibition of myogenesis has to be addressed to a failed recruitment of satellite cells in the muscle fibers (Guttridge et al. 2000; Li and Reid 2001). NF-κB could also act by inducing the degradation of the master gene MyoD, raising the general conclusion that different mediators of muscle atrophy might operate at different stages. The crosstalk of these mediators ensures a fully and complete atrophy program (Guttridge et al. 2000). Trigging signal of atrophy mediated by TNFα may be represented also by p38, able to upregulate MAFbx expression but not MURF1, and acting through an NF-κB independent pathway. By reverse, pharmacological inhibitors of p38 block the atrophic pathway and stabilize the atrogin-1 levels (Li et al. 2005).

Myostatin

Some cattle breeds such as the Belgian Blue display an hypertrophic phenotype with a great increase in muscle mass, such phenotype has been correlated with mutation occurring in the myostatin gene, also named growth and differentiation factor 8 (GDF8) (Charlier et al. 1995). In 2004, Schuelke et al. (2004) described for the first time a mutation of the myostatin gene in humans correlating with an enlargement of the skeletal muscle apparatus. So

myostatin, a member of the TGFβ superfamily, is considered one of the powerful negative regulators of muscle growth, and in mice knockout of myostatin gene leads to a terrific increase of muscle growth (McPherron et al. 1997). In mice, myostatin is predominantly expressed in skeletal muscle, both at 9.5 days post coitum (dpc) and in adulthood; the transcript is located preferentially in red than in white muscles suggesting that myostatin could be involved in the growth balance between fast and slow muscles (McPherron et al. 1997; Roberts and Goetz 2001). Like all TGFβ members, myostatin exerts its activity through the tyrosin-kinase receptors type I and type II, and several studies reveal that probably the Activin Receptor IIB mediates the myostatin action (Lee and McPherron 2001). Following tetramerization of the receptor complex, the signal is relayed into the cytoplasm thanks to SMAD protein phosphorylation (Seuntjens et al. 2009); the regulatory SMAD 2 and 3 binds to SMAD4 and translocate into the nucleus to modify gene expression. In addition, the inhibitory SMAD7, which expression is induced by myostatin, represents a negative feedback loop mechanism by preventing the formation of a correct SMAD complex and blocking myostatin-induced cascade (Massague and Chen 2000; Ebisawa et al. 2001; Zhu et al. 2004). Other proteins are able to interfere with myostatin activity by direct binding, such as the growth and differentiation factor-associated protein-1 (GASP-1), the follistatin related gene (FLRG) or the follistatin, all these proteins basically inhibit muscle atrophy when bound to different myostatin isoforms (mature or immature peptide) (Nakamura et al. 1990; Iemura et al. 1998; Amthor et al. 2002; Hill et al. 2003). Transgenic mice expressing the human isoform of follistatin in muscles display a huge increase in muscle mass comparable to the one observed in myostatin null mice (Lee and McPherron 2001). 6 months old myostatin-null mice show a striking 40% increase of muscle mass compared both to heterozygous and wild type counterpart with a 2- 3-fold increase in muscle weight. On the opposite, transgenic male mice overexpressing myostatin show a 20% decrease in the cross sectional area with a reduced number of nuclei per myofiber. Surprisingly, transgenic female mice are not affected by myostatin overexpression (Reisz-Porszasz et al. 2003). Overall, a mild downregulation of myostatin expression is sufficient to induce muscle hypertrophy but not hyperplasia whereas total myostatin suppression does. The myostatin expression is regulated by many factors as demonstrated by the presence of E-boxes (MyoD binding sites), MEF2 binding sites, GREs and androgen response elements (ARE), suggesting that its activity is required also to control important myogenic events such as myoblast differentiation and proliferation (Ma et al. 2001; Spiller et al. 2002; Crisa et al. 2003; Salerno et al. 2004). In vitro studies on C2C12 demonstrate that myostatin treatment could control cell cycle progression and inhibit myoblast proliferation by upregulating the p21 expression and decreasing the phosphorylation of retinoblastoma 1 protein (pRb); nonetheless myostatin treatment perturbs also the myogenic differentiation events by decreasing MyoD, Myf5 and myogenin levels whereas myostatin silencing leads to a considerable fusion index increment (Langley et al. 2002; Rios et al. 2002; Joulia et al. 2003). Thanks to the ability of modulating muscle mass, myostatin expression could be translated in a therapeutic view in order to improve muscle function in several pathologies. Different experimental strategies have been adopted and most of them used the mdx mice as disease model, containing a nonsense mutation in the dystrophin gene and representing a genetic orthologue of Duchenne and Becker muscular dystrophy with progressive muscle wasting, fiber necrosis and continuous cycles of regeneration/degeneration occurring (Nowak and Davies 2004; Coulton et al. 1988a, b). Double crossing of myostatin-null with mdx mice or systemic injection of blocking antibodies in the dystrophic recipient results in an increased body weight and muscle mass and decreased degeneration rate leading to an attenuation of the dystrophic phenotype (Bogdanovich et al. 2002, 2005 Wagner et al. 2002). Surprisingly, myostatin blockade is not able to rescue the phenotype in another model of muscular dystrophy lacking lamininα2 expression, the dy^w/dy^w mice, claiming that probably myostatin targeting cannot be considered a general treatment for all muscular dystrophies (Li, Shelton et al. 2005). Greatly controversial is the influence of myostatin on satellite cells behavior during adulthood, since few years ago all the studies report that myostatin inhibition positively regulates satellite cells activation. In myostatin-null mice, twofold activation of satellite cells, faster regeneration rate together with a decrease in the inflammatory cells recruitment, fibrosis and fatty infiltration in the injured sites was reported and compared to the cardiotoxin-treated wild type counterpart (Wehling et al. 2000; McCroskery et al. 2003, 2005; Wagner et al. 2005). More recently, these conclusions were strongly criticized by the group of Partridge showing that muscle hypertrophy driven by myostatin blockade not involves satellite cells. According to their results myostatin inhibition doesn't interfere with satellite cells proliferation and hypertrophic fiber contains no more myonuclei or new satellite cells. Moreover, they report a faint expression of myostatin receptor by satellite cell with any apparent contribution of myonuclei from satellite cells in the hypertrophic muscle of dystrophic mice treated with myostatin neutralizing antibodies (Amthor et al. 2006). It is clearly important, before to consider further clinical trials using myostatin blockade, to elucidate its action on satellite cells and especially on the determination of adult muscle stem cells that later

contribute on muscle homeostasis. If our knowledge about myostatin involvement in muscle size and regeneration is continuously growing, less is known about its effect on adipose tissue. In vitro data show that 10T/2 cells treated with azacytidine and recombinant myostatin upregulate the expression of early and late adipogenic markers, and this is consistent with the observation in myostatin-null mice, where serum leptin levels were strongly decreased with a age-dependent reduction of the total fat body mass, up to 70% in 32 weeks old mice (McPherron and Lee 2002; Artaza et al. 2005).

Glucocorticoid

A brief note just to mention that glucocorticoid level are shown to be elevated in muscle wasting conditions and that at molecular level glucocorticoid treatment succeed to upregulate the expression of atrogenes both in vitro and in vivo. At variant, treatment with glucocorticoid receptor antagonists mild muscle loss in some pathological conditions. Still debated is the mechanism through which these compounds act intracellularly and which would be the pathway targeted. No direct regulation of atrogenes expression was described; neither glucocorticoid responsive elements (GRE) were identified on atrogenes promoters. Recently, some reports show a downregulation of IGF-1 and upregulation of myostatin secretion following glucocorticoid treatment, also if loss and gain of function experiments on glucocorticoid receptor needs to be performed in order to better elucidate their direct role in muscle growth. (Sacheck et al. 2004; Sandri et al. 2004; Schakman et al. 2008).

Dystrophin-glycoprotein complex (DGC)

It sounds a bit weird to discuss about DGC in controlling muscle size since 7 years ago it was thought to play a pure structural role by anchoring the cellular cytoskeleton to the sarcomembrane. The members of this complex include at least 12 different gene products, dystrophin, dystroglycans, four sarcoglycans (possibly 5 or 6), sarcospan, syntrophins, nitric oxide synthetase (nNOS) and dystrobrevin. They are membrane-spanning subunits, such as β-dystroglycan and sarcoglycans, as well as strictly intracellular and extracellular components, such as syntrophins, dystrobrevin and α-dystroglycan. Muscle contraction in both heart and skeletal muscle results in cellular deformation and shortening. Throughout this process, the contractile machinery inside the myofibers must remain intimately connected with the membrane and extracellular matrix, thanks to the final binding of α-dystroglican, on the muscle membrane, with laminin-2 located in the extracellular matrix. Without this association, movement would be improperly transmitted and myocytes would risk damage to their membranes. One function of the dystrophin glycoprotein complex (DGC) is to provide a strong mechanical link from the intracellular cytoskeleton to the extracellular matrix (Lapidos et al. 2004). Considerable work has focused on identifying the members of this complex and delineating the functions of the individual parts and the complex as a whole. First data concerning a possible role of this complex in signaling pathways was reported by the group of Rando in 2002, showing that disruption of laminin-2 binding to the dystroglican complex has been able to inhibit AKT activation, one of the master regulator of muscle hypertrophy (Langenbach and Rando 2002). Since laminin is able to stimulate integrin receptors it was unclear whether the molecular mechanisms of AKT inhibition would be accounted by DGC complex or indirectly by the integrin system. A possible solution came from the studies performed by Acharyya et al (2005) on dystrophin expression in cachectic tumor-induced animal models. In atrophic conditions, the authors observed a strong perturbation of the myofibrillar component in skeletal muscle connecting the cachexia tumor-induced with a loss of DGC integrity and the upregulation of muscle wasting became more stressed when the tumors were injected in the mdx animal model, lacking dystrophin expression. If the latter conclusion could be not surprising because mdx muscles are damaged *per se*, the author went deeper in the system by analyzing muscle wasting using an animal model overexpressing dystrophin and they observed that in atrophy conditions the muscle loss was less pronounced compared to both wt and mdx mice concluding that dystrophin expression could help to counteract muscle wasting events. Nonetheless, a strong downregulation of atrogenes such as MAFbx and MURF1 was observed in dystrophin transgenic compared both to wt and mdx mice, let them possible to speculate that if AKT play a key role in downregulating MAFbx and MURF1 and its activity is perturbated by DGC complex, this pathway is the one regulated by dystrophin and its binding proteins. Naturally, direct evidence is missing and the authors raise the possibility of other mechanisms involved including an AKT-independent downregulation of atrogenes. In fact, new reports claim that nNOS, disregulated during muscular dystrophy, targets directly the upregulation of FoxOs transcription factor representing a direct link between dystrophin and muscle atrophy (Suzuki et al. 2007).

Satellite cells and muscle remodeling

Satellite cells can be identified by their position beneath the basal lamina of muscle, closely juxtaposed to muscle fibers (Mauro 1961). They are considered the main progenitors of adult skeletal muscle and present several stem cell

properties (Zammit and Beauchamp 2001; Dhawan and Rando 2005; Zammit et al. 2006). In adult healthy muscle fibers, the percentage of satellite cells is very low around 2,5–6% of fiber nuclei, but once activated they can generate large numbers of new myotubes, within 3–4 days after severe injury (Whalen et al. 1990). Under normal conditions they are quiescent and can be recognized not only by the position, but also by the expression of characteristic markers. One of the most important gene, expressed in the vast majority of quiescent satellite cells and in fetal progenitors of the myogenic lineage, is the paired-box transcription factor Pax7 (Beauchamp et al. 2000; Reimann et al. 2004; Zammit et al. 2006). The addition of new nuclei to existing myofiber during hypertrophic events has been extensively studied but still the mechanism leading to satellite cells activation during this process has not clearly understood. DNA damage experiments induced by γ-ray have been used to test the role of satellite cells in muscle hypertrophy (Rosenblatt et al. 1994; Phelan and Gonyea 1997; Adams et al. 2002). Compensatory muscle enlargement could be partially prevented following γ-irradiation providing a direct evidence that satellite cells are needed to complete the muscle increase; nonetheless since the hypertrophic events are not fully abolished, the possibility of a satellite cells source surviving, an increased protein synthesis in the remaining fibers or the effort by other adult muscle stem cells cannot be excluded (McGeachie and Grounds 1987; Heslop et al. 2000). During mechanical overload or acute/chronic damage the regeneration process activates an inflammatory response with a consequent release of many growth factors involved in the remodeling. Such factors like hepatocyte growth factor (HGF), fibroblast growth factor 6 (FGF6), interleukin-4 (IL-4), interleukin 6 (IL-6), IGF1 play a key role during the activation, proliferation and differentiation events of the satellite cells (Allen et al. 1995; Husmann et al. 1996; Putman et al. 1999; Horsley et al. 2003). Among these, IGF1 seems to be the most important factor linking muscle hypertrophy and satellite cells, muscle hypertrophy IGF1-dependent is only slightly reduced following γ irradiation and prevent the aging-related muscle wasting thanks to an improved regenerative potential of satellite cells (Barton-Davis et al. 1999; Musaro et al. 2001). Furthermore, IGF1 increases the DNA content per myofiber, the centronucleated fiber and the expression of different myosin isoform; satellite cells isolated from IGF1 overexpressing mice display an increased regenerative potential compared to wt counterpart, which is mediated by FoxO1 and the activity of the $p27^{kip1}$ (Adams and McCue 1998; Chakravarthy et al. 2000; Fiorotto et al. 2003; Machida et al. 2003). By another part, Notch and IGF1 are involved in the ageing process of satellite cells with a consequent loss of muscle weight and physical performance, in fact manipulations of Notch and IGF1 pathways result in the activation and proliferation of satellite cells and may provide a good tool to slowdown the age-related muscle wasting (Conboy and Rando 2002; Conboy et al. 2003; Sherwood et al. 2004).

microRNA and muscle remodeling

microRNA (miRNA) are small non-coding RNA transcripts, ~22 nucleotides long able to inhibit translation or promote mRNA degradation (common in plants) by annealing to complementary sequences in the 3′untranslated regions (UTR) of specific target mRNA. Individual miRNA can target dozen of transcripts based of the specificity of the seed sequence (8 nucleotides) located to the 5′ of miRNA. miRNAs can also influenced the expression of other miRNAs, unraveling an enormous complexity and regulatory potential for gene expression (Bartel 2004; Stark et al. 2005; Lee et al. 2007). Recent studies showed another regulation step in muscle growth and differentiation mediated by a collection of muscle specific miRNA, that could be involved in fine tuning the expression of master genes in muscle development such as Pax3, MyoD and other cardiac and skeletal muscle specific factors. Muscle specific deletion of Dicer, a critical component of miRNA machinery, has deep effect on embryonic and postnatal development, revealing a key role of miRNAs in controlling muscle development (O'Rourke et al. 2007). miR-206 is the most abundant miRNA in adult vertebrate skeletal muscle and play an important role in muscle plasticity and remodeling. It has been demonstrated that miRNA206 promotes muscle differentiation and is strongly upregulated during muscle hypertrophy induced by surgical removal of gastrocnemius (synergistic ablation). In this condition the expression level of pre-miRNA206 is 18-fold higher than the control, with a correspondent downregulation of miRNA1 and 133, both involved in skeletal muscle development (McCarthy et al. 2007). Loss of function studies revealed that miRNA206 knockdown through antisense oligonucleotides blocks myoblasts from exiting the cell cycle inhibiting muscle differentiation by a negative regulation of the DNA polymerase α. Moreover, miRNA206 could interfere with satellite cells behavior, since among its predicted target there are cMet and Pax3 essential for satellite cells migration and early myogenesis steps. This suggests that regulation of gene expression by this miRNA is crucial for early and late muscle homeostasis. Nonetheless, a mutation that causes a dramatic muscle increase in Texel sheep has been mapped in the myostatin gene, and this mutation creates a target site for miR-206 and miR-1 resulting in myostatin downregulation, developing a phenocopy of the double-muscled cattle previously described in Myostatin section (Clop et al. 2006). Our knowledge of miRNA

biology is still in its infancy and future investigation need to be done for clarifying the molecular mechanism and the precise involvement of these miRNA in muscle size control.

Future perspectives

Different molecules are identified so far to interact with biochemical machinery controlling muscle mass. Several scientists investigate about the use of small molecules that interfere with the activity of histone deacetylase and are now under clinical investigation. The transcriptional activity of FOXO proteins is negatively regulated by Sir2 overexpression and further studies will address the efficacy of resveratrol, a Sir2 agonist, in abolishing the atrophic program driven by FOXO activation (Aziz et al. 2003; Secrist et al. 2003; Motta et al. 2004; Brunet et al. 2004). Deacetylase inhibitors, such as valproic acid, represent a powerful tool in size remodeling and muscle atrophy prevention by mainly activating follistatin expression (Minetti et al. 2006). Future experiments will determine if these small drugs could target the progression of muscle wasting in animal model of cachexia-related diseases. Interestingly, resveratrol treatment interferes with AKT activity as well as the other therapeutic approaches to induce muscular hypertrophy. While addressing the molecular mechanisms leading to reprogramming will provide new insights into the factors conferring plasticity to differentiated nuclei, it is also important to verify these results in more relevant contexts, such as primary cells and in vivo studies. Magic-F1 is an HGF-derived molecule with a potential clinical application as it can induce muscle hypertrophy by activating MyoD and Myf5, and preventing apoptotic events (Cassano et al. 2008). Interestingly, no side effects have been observed in skeletal muscles following electro-enhanced Magic-F1 DNA transfer or in transgenic mice expressing Magic-F1 under the control of a muscle-specific promoter. Magic-F1 is not able to induce the ERK pathway and this fact is particular relevant to a potential therapeutic use of this engineered factor. In this regard, the lack of any mitogenic activity makes Magic-F1 a potentially safe cytokine for cell therapy. Recently, it has been reported that HGF gene therapy improves LV remodeling and dysfunction post-infarction through promotion of cardiomyocyte hypertrophy, and that HGF plays a role in the induction of stem cell commitment to the cardiomyocyte lineage (Fiaccavento et al. 2005; Forte et al. 2006; Li et al. 2003). Magic-F1 exhibits biological effects in the renewal of skeletal muscles tissues similar though not identical to those observed for HGF in cardiac tissue regeneration. Further studies are necessary to elucidate the different potential effects of HGF in this context and—in this sense—supplementary studies on Magic-F1 signal transduction could provide useful information. Given the small size of its cDNA (approximately 1.7 kb), Magic-F1 may be used alone in a gene therapy setting or inserted as a second adjuvant transgene in a vector encoding a therapeutic gene or an anti-cachectic factor (hystone acetylase inhibitor) (Minetti et al. 2006). Recent works demonstrated the efficacy of pericytic-derived cells, named mesoangioblast, directly contributing to muscle regeneration in dystrophic conditions (Sampaolesi et al. 2003, 2006; Galvez et al. 2006; Gargioli et al. 2008). In this context, Magic-F1, as adjuvant transgene, could positively affect homing ability and regeneration potential of mesoangioblasts. Finally, there are evidences indicating that miRNAs may have an important role in muscle hypertrophy. In fact, miR-1 cluster, miR-133 and miR-206 are involved in muscle growth and regeneration. The challenge for future studies will be to better identify the relevant target genes of muscle-specific miR and how they can be used alone or in combination with the other molecules or delivered by stem cells in order to contribute to the improvement of skeletal muscle growth and strength.

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